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Pharmacological Properties of Two Cloned Somatostatin Receptors

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SUMMARY

Previous studies have shown that at least two subtypes of somatostatin (SRIF) receptors ($SRIF_1$ and $SRIF_2$) are expressed in mammalian cells. $SRIF_1$ receptors have high affinity for MK 678, whereas $SRIF_2$ receptors have no affinity for MK 678 but selectively bind peptides with structures similar to that of CGP 23996. Recently, two SRIF receptor genes have been cloned from human and mouse genomic libraries. In the present study, the pharmacological properties of these two cloned SRIF receptors, expressed in Chinese hamster ovary (CHO) cells, were investigated, to determine whether they have any similarity to the previously described $SRIF_1$ and $SRIF_2$ receptor subtypes. Both cloned receptors could be labeled with ^{125}I-Tyr^{11}-SRIF and exhibited high affinity for SRIF. The SSTR1 receptor could also bind CGP 23996-like compounds but not MK 678. In contrast, the SSTR2 receptor was insensitive to CGP 23996-like compounds but bound MK 678 with high affinity. These findings indicate that the peptide specificities of the cloned SSTR1 and SSTR2 receptors differ from each other. Pretreatment of CHO cells expressing the two cloned SRIF receptors with SRIF abolished high affinity agonist binding to the cloned SSTR2 receptor but not the cloned SSTR1 receptor. Agonist binding to SSTR1 receptors was not significantly affected by guanosine-5′-*O*-(3-thiotriphosphate) or pertussis toxin pretreatment, whereas agonist binding to SSTR2 receptors was inhibited by both treatments. These findings suggest that SSTR2 receptors can be regulated and they associate with pertussis toxin-sensitive guanine nucleotide-binding proteins, whereas SSTR1 receptors do not. SRIF is a potent inhibitor of adenylyl cyclase activity in mammalian cells. However, neither the cloned SSTR2 nor SSTR1 receptor mediated SRIF inhibition of adenylyl cyclase activity in stably transformed CHO cells or COS-1 cells transiently expressing the cloned receptors, suggesting that neither cloned receptor couples to adenylyl cyclase. The results of these studies indicate that the two cloned SRIF receptors have different pharmacological properties. The characteristics of the cloned SSTR2 receptor are similar to those of the previously described $SRIF_1$ receptor, and the characteristics of the cloned SSTR1 receptor are similar to those of the previously described $SRIF_2$ receptor.

The neuropeptide SRIF is an important regulator of endocrine and brain function (1, 2). It is the major physiological inhibitor of growth hormone secretion from the pituitary and of insulin and glucagon release from pancreatic islets (1, 2). In brain, it acts as a neurotransmitter/neuromodulator to inhibit neuronal firing (3, 4) and has a role in the modulation of complex behaviors such as motor activity and cognition (5, 6).

SRIF induces its biological actions by interacting with membrane-bound receptors. These receptors are coupled by pertussis toxin-sensitive G proteins to adenylyl cyclase (7, 8) and ionic conductance channels (9, 10). SRIF has been shown to be a potent inhibitor of adenylyl cyclase activity (7, 8) and Ca^{2+} conductance (9) and can potentiate K^+ currents (10).

Recent pharmacological studies have distinguished at least two subtypes of SRIF receptors in brain and other tissues (11–13). One receptor exhibits high affinity for the cyclohexapeptide MK 678 and its structural analogs and has been referred to as the $SRIF_1$ receptor (13, 14). The other SRIF receptor subtype has no affinity for MK 678 but has high affinity for SRIF and SRIF-28 and can selectively bind c[Ahep-Phe-D-Trp-Lys-

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ABBREVIATIONS: SRIF, somatostatin; $SRIF_1$ receptor, somatostatin$_1$ receptor; $SRIF_2$ receptor, somatostatin$_2$ receptor; CHO cells, Chinese hamster ovary cells; G protein, guanine nucleotide-binding regulatory protein; G_i, the inhibitory guanine nucleotide-binding protein; G_o, the predominant pertussis toxin-sensitive guanine nucleotide-binding protein in brain; SSTR1 receptor, cloned somatostatin receptor containing 391 amino acids; SSTR2 receptor, cloned somatostatin receptor containing 369 amino acids; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N, N, N′, N′*-tetraacetic acid; GTPγS, guanosine-5′-*O*-(3-thiotriphosphate).

Thr(Bzl)] (compound 1), c[Ahep-Phe-D-Trp-Lys-Ser(Bzl)] (compound 2), and c[Ahex-Phe-D-Trp-Lys-Thr(Bzl)] (compound 3), which are structural analogs of the SRIF agonist CGP 23996 (14). This receptor subtype has been named the $SRIF_2$ receptor (13, 14). These receptor subtypes have different regional distributions in rat brain (11, 15) and may mediate selective behavioral effects of SRIF (16).

Comparison of the biochemical characteristics of the two receptors has been limited. The $SRIF_1$ receptor can be labeled with high affinity by ^{125}I-MK 678 and can be solubilized and partially purified (17). This receptor is glycosylated, and sialic acid residues in the receptor are necessary for high affinity agonist binding to the receptor (18). Furthermore, the $SRIF_1$ receptor has been shown to interact selectively with G protein α subunits $G_{i\alpha1}$, $G_{i\alpha3}$, and $G_{o\alpha}$ in brain and pituitary cells (19, 20), and $G_{i\alpha1}$ has been shown to couple selectively the $SRIF_1$ receptor to adenylyl cyclase in the pituitary cell line AtT-20 (21). Antibodies have been generated against the $SRIF_1$ receptor and, using these antibodies, its size has been estimated to be 60 kDa (22). In contrast, no radioligand can selectively label the $SRIF_2$ receptor with high affinity, and this receptor subtype has not been successfully solubilized. Therefore, its size and physical properties are not known. The lack of this information has hindered attempts to understand the physical basis for the different pharmacological and functional properties of these two SRIF receptor subtypes.

Recently, the genes encoding two SRIF receptor subtypes (SSTR1 and SSTR2) were cloned (23). The predicted amino acid sequences of the two receptors are different, as are the tissue distributions of their mRNAs. The SSTR1 and SSTR2 genes were stably expressed in CHO cells and shown to encode receptors that could be detected with ^{125}I-Tyr^{11}-SRIF and that exhibited high affinity for SRIF and SRIF-28. In the present study, we investigated whether the pharmacological properties of the two cloned receptors differed and whether they may correspond to the previously described $SRIF_1$ and $SRIF_2$ receptor subtypes. Our results indicate that one of the cloned receptors (SSTR2) has pharmacological characteristics similar to those of the $SRIF_1$ receptor, whereas the properties of the other cloned receptor (SSTR1) are similar to those of the $SRIF_2$ receptor.

Experimental Procedures

SRIF, SRIF-28, and compound 1 were obtained from Bachem (Torrance, CA). Compounds 2 and 3 were gifts from Dr. D. Coy (Tulane University, New Orleans, LA). MK 678 and MK 301 were gifts from Dr. D. Veber (Merck, West Point, PA), SMS-201-995 was obtained from Sandoz (Basel, Switzerland), and CGP 23996 was a gift from Dr. B. Petrack (Ciba Geigy, Rahway NJ). ^{125}I-Tyr^{11}-SRIF (specific activity, 2000 Ci/mmol) was obtained from Amersham (Arlington Heights, IL), and MK 678 was iodinated as previously described (11). GTPγS was obtained from Boehringer-Mannheim (Indianapolis, IN). Pertussis toxin was obtained from List Biologicals (Campbell, CA).

To generate the CHO cells (cell line DG44) expressing the SSTR1 and SSTR2 receptors, a 1.5-kilobase *PstI*/*XmnI* fragment of the human SSTR1 gene or a 1.2-kilobase *XbaI* fragment of the mouse SSTR2 gene was inserted into the expression vector pCMV6b or -6c, respectively, and co-transfected with pSV2neo into CHO cells, as previously described (23). Stable transfectants were selected and grown as described (23). The cloned receptors were also transiently expressed in COS-1 cells, using a previously described procedure (24). For these studies, cells plated in either 12-well plates or T75 flasks were exposed for 24 hr to the vectors containing the receptor genomic fragments. The cells were then washed and maintained in growth medium for 24 hr, and then receptor binding studies or cAMP studies were performed.

For the receptor binding studies (11, 12), CHO cells stably expressing the receptors or COS-1 cells transiently expressing the receptors were detached from the culture flasks, and the cells were homogenized in a buffer consisting of 50 mM Tris (pH 7.4) containing 1 mM EGTA, 5 mM $MgCl_2$, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 200 μg/ml bacitracin, and 0.5 μg/ml aprotinin (buffer 1), using a Brinkman Polytron (setting 2, 15 sec). The homogenates were centrifuged at 45,000 $\times$ *g* for 15 min at 4°, and the pellet was resuspended in buffer 1 and used in the radioligand binding assay. For the binding assay, membranes were incubated in buffer 1 with either ^{125}I-Tyr^{11}-SRIF (specific activity, 2000 Ci/mmol; 60,000 cpm) or ^{125}I-MK 678 (2200 Ci/mmol; 100,000 cpm), in the presence or absence of unlabeled peptides or GTPγS, in a total volume of 200 μl, for 30 min (^{125}I-Tyr^{11}-SRIF) or 90 min (^{125}I-MK 678) at 25°. The binding reaction was terminated by vacuum filtration over Whatman GF/F glass fiber filters that had been presoaked in 0.5% polyethyleneimine (w/v) and 0.1% bovine serum albumin, using a Millipore filtration apparatus. The filters were washed with 15 ml of ice-cold Tris·HCl (pH 7.8), and the bound radioactivity was analyzed in a γ-counter (80% efficiency). Specific binding was defined as the total ^{125}I-Tyr^{11}-SRIF or ^{125}I-MK 678 binding minus the amount bound in the presence of 100 nM SRIF. Data from these studies were used to generate inhibition curves. IC_{50} values were obtained from curve-fitting performed by the mathematical modeling program FITCOMP (available on the NIH-sponsored PROPHET System (14)).

To measure the effect of SRIF agonists on cAMP formation in CHO cells or COS-1 cells, procedures similar to those previously described (18) were used. Briefly, CHO cells were preincubated with minimum essential medium containing 500 μM isobutylmethylxanthine. The cells were washed and then exposed to similar medium containing either forskolin (50 μM) or forskolin plus SRIF (1 μM), for 30 min at 37° in 10% CO_2. The reaction was terminated by removing the medium, adding 1 N HCl to the cells, sonicating the cells, removing the samples from the plates, evaporating the acid in a SpeedVac concentrator (Savant Instruments, Inc., Farmingdale, NY), and analyzing the cAMP content of the cells by using a commercially available (NEN) cAMP radioimmunoassay. In these studies, forskolin induced a 6-fold stimulation of cAMP formation in these cells.

Immunoblotting to detect α subunits of G proteins in CHO or COS-1 cell membranes was performed as previously described (20). The peptide-directed antisera 3646 (anti-$G_{i\alpha1}$), 1521 (anti-$G_{i\alpha2}$), 1518 (anti-$G_{i\alpha3}$), and 9072 (anti-$G_{o\alpha}$) (at 1/100 dilutions) were used to detect the different G proteins. The specificities of these antisera have been described extensively elsewhere (20).

Results

To investigate the pharmacological properties of the two cloned SRIF receptors, the genes encoding the two cloned receptors were stably expressed in CHO cells. To characterize the properties of the expressed receptors, each receptor subtype was labeled with ^{125}I-Tyr^{11}-SRIF, which binds to all SRIF receptors. ^{125}I-Tyr^{11}-SRIF binding to SSTR1 and SSTR2 receptors reached equilibrium by 30 min and was potently inhibited by SRIF, with IC_{50} values of 2.1 and 0.08 nM, respectively (Fig. 1, A and B). No specific ^{125}I-Tyr^{11}-SRIF binding was detected in membranes of nontransformed CHO cells (data not shown). The B_{max} value for ^{125}I-Tyr^{11}-SRIF binding to SSTR1 and SSTR2 receptors obtained from saturation analysis was 990 fmol/mg of protein and 262 fmol/mg of protein, respectively. The $SRIF_2$ receptor-selective peptide compound 1 (14) completely displaced ^{125}I-Tyr^{11}-SRIF binding to SSTR1 receptors but did not interact with SSTR2 receptors (Fig. 1, A and B). Similarly, two structural analogs of this peptide, compound 2 and compound 3, which selectively bind to $SRIF_2$ receptors

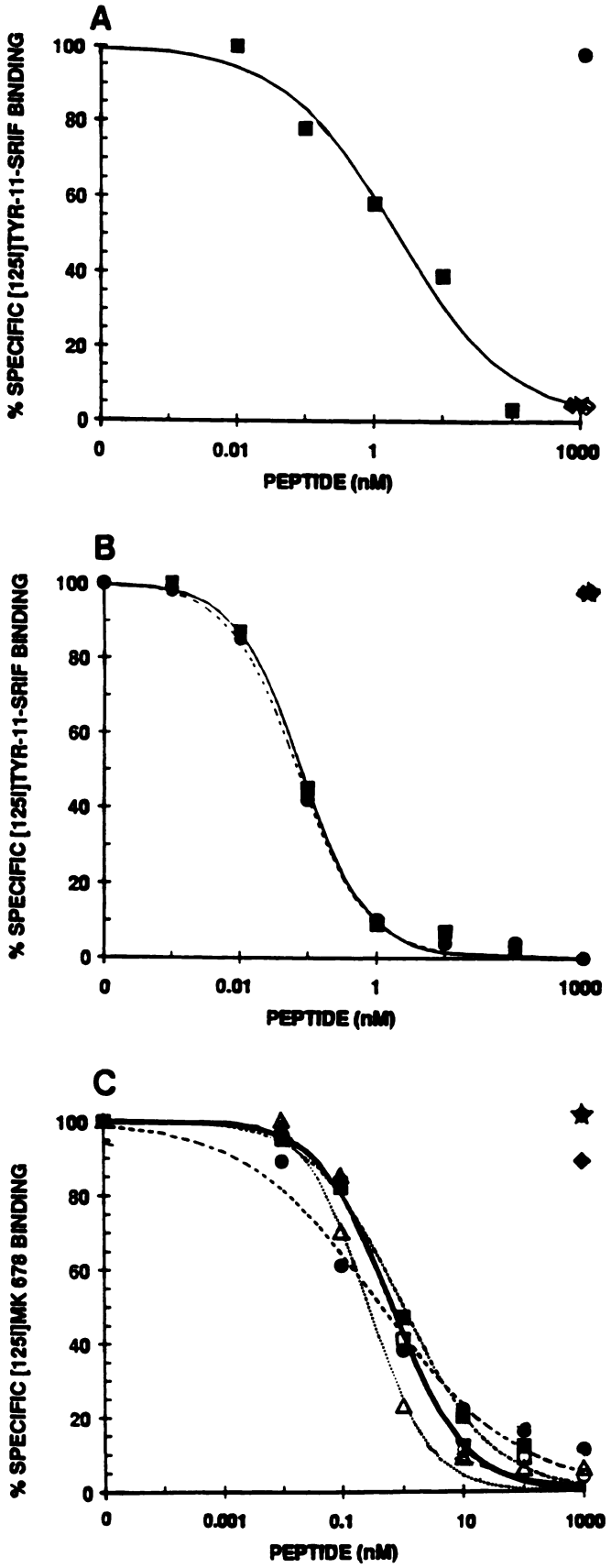

Fig. 1. Pharmacological specificities of the cloned SSTR1 and SSTR2 receptors. The binding of ^{125}I-Tyr^{11}-SRIF (50 pM) to the cloned SSTR1 receptor (A) and to the cloned SSTR2 receptor (B) was measured as described in Experimental Procedures. The ability of SRIF (■), MK 678 (●), compound 1 (♦), compound 2 (◇), and compound 3 (★) to inhibit the binding of ^{125}I-Tyr^{11}-SRIF to the cloned receptors was tested. These results are the means of three different determinations. The ability of SRIF (□), SRIF-28 (△), CGP 23996 (■), SMS 201-995 (●), compound 1 (♦), compound 2 (◇), and compound 3 (★) to inhibit the binding of ^{125}I-MK 678 (80 pM) to the cloned SSTR2 receptor was tested (C). The IC_{50} values for the peptides to inhibit ^{125}I-MK 678 binding are SRIF, 0.67 nM; SRIF-28, 0.27 nM; CGP 23996, 1.0 nM; and SMS 201–995, 0.5 nM. These are the means of three different experiments.

(14), interacted with SSTR1 receptors but not SSTR2 receptors (Fig. 1, A and B). MK 678, which specifically binds to $SRIF_1$ receptors (11–14), potently displaced ^{125}I-Tyr^{11}-SRIF binding to SSTR2 receptors, with an IC_{50} value of 0.07 nM, but did not affect ^{125}I-Tyr^{11}-SRIF binding to SSTR1 receptors (Fig. 1, A and B). These findings indicate that the cloned SRIF receptors have distinct pharmacological characteristics.

Previous studies have shown that ^{125}I-MK 678 binds with high affinity and selectively to $SRIF_1$ receptors (11–14). No specific ^{125}I-MK 678 binding to SSTR1 receptors could be detected, consistent with the inability of MK 678 to inhibit ^{125}I-Tyr^{11}-SRIF binding to SSTR1 receptors. However, ^{125}I-MK 678 potently bound to SSTR2 receptors (Fig. 1C). SRIF and SRIF-28 had similar potencies to inhibit ^{125}I-MK 678 binding to SSTR2 receptors (Fig. 1C). Furthermore, the stable SRIF agonists SMS-201–995 and CGP 23996, which can bind to all SRIF receptors at high concentrations, potently inhibited ^{125}I-MK 678 binding to SSTR2 receptors. These findings indicate that the pharmacological characteristics of the mouse SSTR2 receptor expressed in CHO cells are similar to those of the $SRIF_1$ receptors endogenously expressed in brain and other tissues.

Previous studies have shown that SRIF receptors in brain, pancreas, and the pituitary cell lines AtT-20 and GH_3 desensitize when chronically exposed to agonists (25–29). Exposure of CHO cells expressing the SSTR2 receptors to 100 nM SRIF or MK 678 for 1 hr greatly decreased the specific binding of ^{125}I-MK 678 to this cloned SRIF receptor (Fig. 2b). The decrease in binding is not due to the presence of excess SRIF agonist associated with the receptor, because washing conditions were used that removed all residual SRIF agonist (25, 27, 28). In contrast to the desensitization of SSTR2 receptors, SSTR1 receptors did not desensitize (Fig. 2a). These findings indicate that the two cloned SRIF receptors can be differentially regulated.

SRIF receptors are complexed with G proteins, and the dissociation of this complex, induced by GTP analogs, converts the receptor into a low affinity state for agonists and has been shown to abolish high affinity agonist binding to SRIF receptors (8, 11, 20). The stable GTP analog GTPγS (100 μM) inhibited ^{125}I-MK 678 binding to SSTR2 receptors, suggesting that this receptor is coupled to G proteins (Fig. 3a). The effect of GTPγS was concentration dependent, with half-maximal inhibition of ^{125}I-MK 678 binding to SSTR2 receptors occurring at 0.8 μM. In contrast, GTPγS at a high concentration (100 μM) did not affect ^{125}I-Tyr^{11} SRIF binding to SSTR1 receptors (Fig. 3a). These findings indicate that the nature of the interaction of the cloned SRIF receptors with G proteins differs.

Previous studies have shown that SRIF receptors couple to their cellular effector systems via pertussis toxin-sensitive G proteins (7–10) and that pertussis toxin uncouples SRIF receptors from G proteins, reducing the affinity of the receptors for

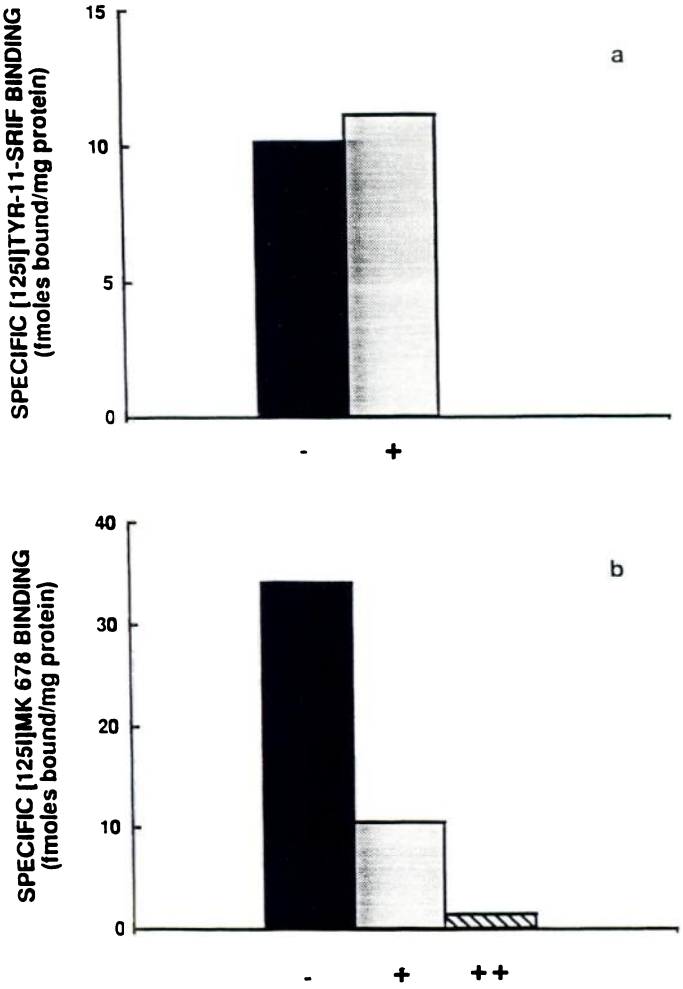

Fig. 2. Differential regulation of the cloned SSTR1 and SSTR2 receptors by SRIF agonists. CHOR1 (a) and CHOR2 (b) cells were treated with (+) or without (−) 100 nM SRIF for 1 hr. A separate group of CHOR2 cells were also treated with 100 nM MK 678 for 1 hr (++). At the end of the treatment, the cells were washed twice with Hanks' balanced salt solution, detached from the flasks, homogenized, centrifuged, and used in the receptor binding assays. SSTR1 receptors were labeled with ^{125}I-Tyr^{11}-SRIF (50 pM), and SSTR2 receptors were labeled with ^{125}I-MK 678 (80 pM). These results are the means of three different experiments. The standard error is <10% of the means.

agonists (8). Pretreatment of CHO cells expressing SSTR2 receptors with pertussis toxin resulted in a complete loss of specific high affinity ^{125}I-Tyr^{11}-SRIF binding to SSTR2 receptors (Fig. 3b). In contrast, similar pretreatment of CHO cells expressing SSTR1 receptors with pertussis toxin did not affect agonist binding to the SSTR1 receptor (Fig. 3b). These findings indicate that the SSTR2 receptor, like the SRIF$_1$ receptor, is coupled to pertussis toxin-sensitive G proteins. In contrast, the cloned SSTR1 receptor does not appear to associate with pertussis toxin-sensitive G proteins in CHO cells.

Pertussis toxin-sensitive G proteins couple SRIF receptors to adenylyl cyclase, and SRIF is a potent inhibitor of cAMP formation (2, 7, 8, 13, 27, 28). However, SRIF agonists were not able to inhibit forskolin-stimulated cAMP formation or adenylyl cyclase activity in CHO cells expressing either cloned SRIF receptor (data not shown). This may be due to the lack of expression of the appropriate G protein needed to couple SRIF receptors to adenylyl cyclase in CHO cells. Recent studies have shown that $G_{i\alpha1}$ is necessary to couple SRIF receptors to adenylyl cyclase in the pituitary cell line AtT-20 (21). Investigation of the G proteins present in both transfected CHO cell lines by immunoblotting, using peptide-directed antisera against different G_i or G_o α subunits, revealed the presence of $G_{i\alpha3}$ but no detectable $G_{i\alpha1}$, $G_{i\alpha2}$, or $G_{o\alpha}$ (Fig. 3c). The lack of significant levels of $G_{i\alpha1}$ may be the cause of the inability of SRIF agonists to inhibit adenylyl cyclase activity in the transformed CHO cells. However, transient expression of $G_{i\alpha1}$ in CHO cells stably expressing either cloned SRIF receptor did not result in SRIF being able to inhibit forskolin-stimulated cAMP accumulation (data not shown). Furthermore, transient expression of the cloned SRIF receptors in COS-1 cells, which express all the subtypes of $G_{i\alpha}$ (based on immunoblotting experiments), resulted in the expression of both cloned receptors, which could be labeled specifically by ^{125}I-Tyr^{11}-SRIF and which exhibited the same pharmacological characteristics as the cloned receptors stably expressed in CHO cells (data not shown). However, as seen with the transformed CHO cells, the COS-1 cells expressing the cloned SRIF receptor did not mediate SRIF inhibition of forskolin-stimulated cAMP formation, suggesting that the cloned receptors do not effectively couple with adenylyl cyclase.

Discussion

The predicted amino acid sequences of SSTR1 and SSTR2 differ considerably from the sequences of any previously cloned receptor, suggesting that these receptors are members of a unique subfamily of G protein-linked receptors (23). Comparison of the amino acid sequence of SSTR1 and SSTR2 indicates that there is 49% overall identity and 64% similarity and there is >85% sequence similarity in the predicted membrane-spanning regions (23). However, the two cloned SRIF receptors have considerable differences in amino acid sequence in the amino- and carboxyl-terminal regions (23). Such structural diversity is likely to provide the basis for functional differences between the SRIF receptor subtypes. The present study provides the first evidence that the pharmacological characteristics of the two cloned receptors differ.

Although both cloned receptors bind SRIF with high affinity, the SSTR1 receptor is unable to bind the superactive SRIF agonist MK 678. In contrast, this peptide potently interacted with the SSTR2 receptor. Furthermore, three peptides (compounds 1–3) that have been previously shown to interact specifically with SRIF$_2$ receptors (14) selectively bind to SSTR1 receptors. These findings suggest that the two cloned receptors display differing pharmacological specificities for SRIF analogs. Because MK 678 specifically binds to SRIF$_1$ receptors (11–14) and compounds 1–3 selectively interact with SRIF$_2$ receptors (14) endogenously expressed in brain and other tissues, our findings suggest that the cloned SSTR1 and SSTR2 receptors have pharmacological characteristics in common with SRIF$_2$ and SRIF$_1$ receptors, respectively (see Table 1 for comparison of IC_{50} values).

Another characteristic that distinguishes SRIF receptor subtypes is their susceptibility to agonist-induced desensitization. SRIF receptors in brain neurons (26) and the pituitary cell line AtT-20 (27, 28) rapidly desensitize when exposed to agonists. In contrast, SRIF receptors in GH_4C_1 cells (30) and rat pituitary (31) are very resistant to desensitization. Recent studies have shown that exposure of SRIF$_1$ receptors in GH_3 cells to SRIF analogs abolishes high affinity agonist binding to the receptor,

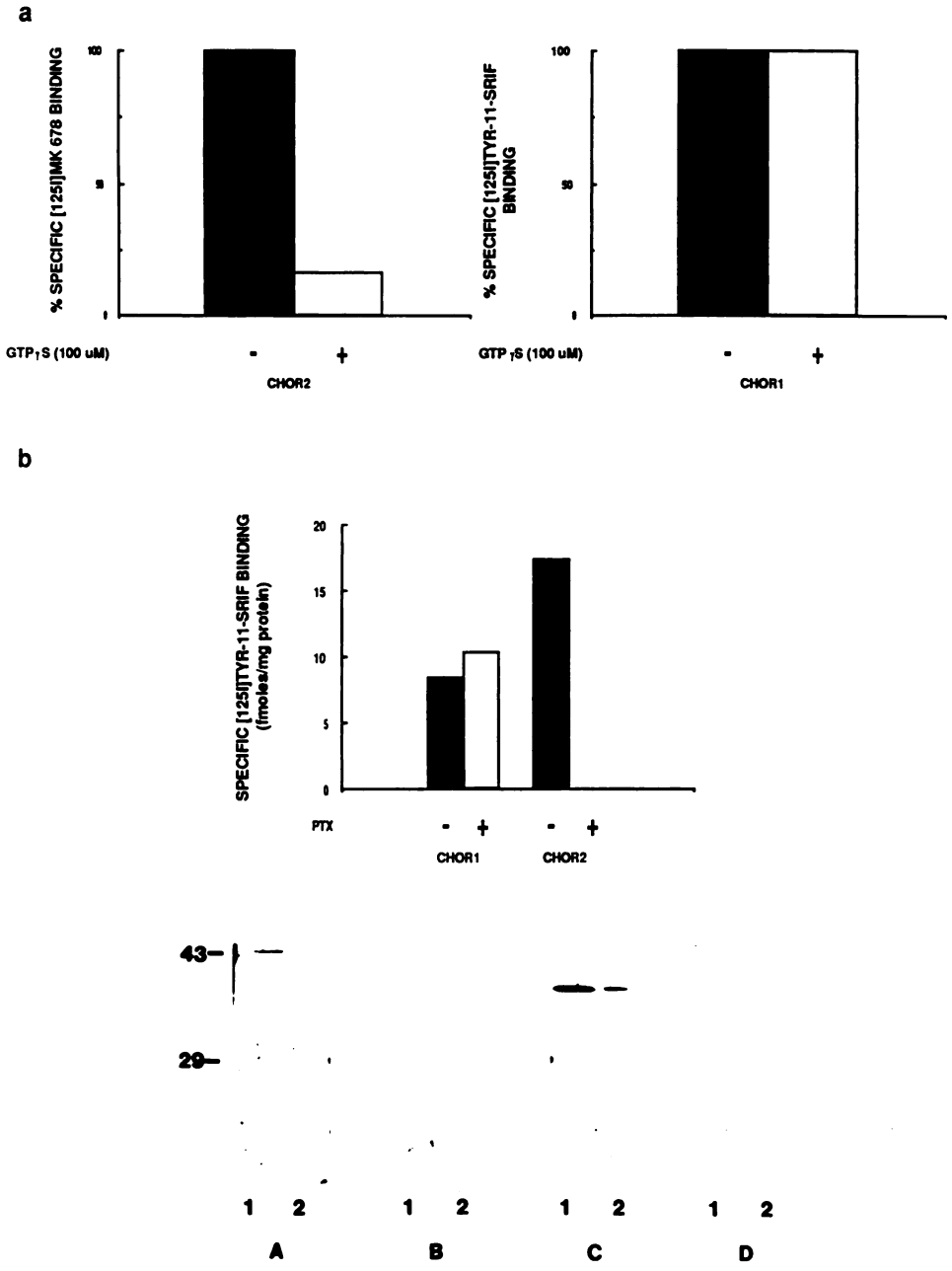

Fig. 3. G protein coupling to the cloned SSTR1 and SSTR2 receptors. a, The effect of GTPγS on the binding of ^{125}I-MK 678 to SSTR2 receptors in CHOR2 cell membranes and on the binding of ^{125}I-Tyr^{11}-SRIF to SSTR1 receptors in CHOR1 cell membranes was measured. Total and nonspecific ^{125}I-MK 678 binding to SSTR2 receptors was 3708 cpm and 454 cpm, respectively. Total and nonspecific ^{125}I-Tyr^{11}-SRIF binding to SSTR1 receptors was 3843 cpm and 1952 cpm, respectively. b, The effect of pretreatment of CHOR1 and CHOR2 cells with pertussis toxin (*PTX*) on the subsequent specific binding of ^{125}I-Tyr^{11}-SRIF to the SSTR1 and SSTR2 receptors, respectively, was measured. Cells were treated with either control medium (■) or pertussis toxin (100 ng/ml) (□) for 24 hr. Note that no specific ^{125}I-Tyr^{11}-SRIF binding to membranes from CHOR2 cells that had been treated with pertussis toxin could be detected. These results are the means of three different experiments, and the standard error of each value is <10% of the mean. c, G proteins present in CHOR1 (*1*) and CHOR2 (*2*) cell membranes were measured by immunoblotting using antisera 3646 ($G_{i\alpha1}$ selective) (*A*), 1521 ($G_{i\alpha2}$ selective) (*B*), 1518 ($G_{i\alpha3}$ selective) (*C*), and 9072 ($G_{o\alpha}$ selective)(*D*). Immunoblotting was performed as described previously (20). Approximately twice as much protein from CHOR1 cells as from CHOR2 cells was added to the lanes. Molecular mass markers (kDa) are at the *left*.

TABLE 1

Comparison of affinities of SSTR1, SSTR2, SRIF$_1$, and SRIF$_2$ receptors for different SRIF analogs

IC_{50} values for peptide binding to SRIF$_1$ and SRIF$_2$ receptors were obtained from Refs. 11 and 14.

Peptide	IC_{50} value			
	SSTR1	SRIF$_2$	SSTR2	SRIF$_1$
	nM			
SRIF	2	3	0.1	2
MK 678	>1,000	>10,000	0.1	2
Compound 1	<1,000	2,000	>10,000	>10,000
Compound 2	<1,000	9,000	>10,000	>10,000
Compound 3	<1,000	2,000	>10,000	>10,000

whereas SRIF$_2$ receptors are less affected by agonist treatment (25, 32). Similarly, SSTR2 receptors expressed in CHO cells desensitize when exposed to SRIF, whereas SSTR1 receptors do not. These findings indicate that the two cloned receptors, like SRIF$_1$ and SRIF$_2$ receptors, can be differentially regulated.

A major difference between the two cloned SRIF receptors is their interaction with G proteins. SRIF receptors endogenously expressed in mammalian cells are known to couple to G proteins, and G protein coupling can be disrupted by GTPγS (2, 7, 8). Consistent with these findings, high affinity agonist binding to SSTR2 receptors was abolished by GTPγS. In contrast, GTPγS did not affect ^{125}I-Tyr^{11}-SRIF binding to SSTR1 receptors. Similar results were observed in studies comparing the GTP sensitivities of SRIF$_1$ and SRIF$_2$ receptors in brain, in which GTPγS abolished high affinity agonist binding to SRIF$_1$ receptors but did not affect agonist binding

to $SRIF_2$ receptors (14). The inability of GTPγS to affect agonist binding to $SRIF_2$ receptors may be due to this receptor, as well as the cloned SSTR1 receptor, not being efficiently coupled to G proteins.

Pretreatment of the transformed CHO cells with pertussis toxin abolished high affinity agonist binding to SSTR2 receptors but not SSTR1 receptors. Previous studies have shown that $SRIF_1$ receptors expressed in brain and other tissues are coupled to pertussis toxin-sensitive G proteins (13, 20). The effects of pertussis toxin on the cloned SSTR2 receptors are similar to what has been observed with the $SRIF_1$ receptor and support the hypothesis that the receptors have similar pharmacological properties. There are no reports on the sensitivity of $SRIF_2$ receptors to pertussis toxin treatment or on whether these receptors couple to pertussis toxin-sensitive G proteins. $SRIF_2$ receptor insensitivity to GTPγS (14), as well as our findings that the cloned SSTR1 receptor is insensitive to GTPγS and pertussis toxin pretreatment, suggests that these two receptors may be very similar.

Although the cloned SSTR1 and SSTR2 receptors have many of the same characteristics as the $SRIF_2$ and $SRIF_1$ receptors, respectively, no evidence exists that these cloned receptors mediate biological actions of SRIF. SRIF was not able to inhibit cAMP formation in the CHO cells stably expressing the receptors. This may be due to the lack of expression, in CHO cells, of $G_{i\alpha 1}$, which has been reported to mediate SRIF inhibition of adenylyl cyclase activity in AtT-20 cells (21). CHO cells have been reported by other investigators (33) to lack $G_{i\alpha 1}$ and this finding was confirmed in our own experiments. Furthermore, CHO cells (DG44 cell line) also lack $G_{o\alpha}$, which couples with $SRIF_1$ receptors, and $G_{i\alpha 2}$, which does not (20). They do express $G_{i\alpha 3}$, which couples to $SRIF_1$ receptors and which may associate with the cloned SSTR2 receptor in CHO cells to maintain the high affinity of the receptor for agonists. However, transient expression of $G_{i\alpha 1}$ in CHO cells stably expressing the cloned receptors did not facilitate SRIF inhibition of forskolin-stimulated cAMP formation. Furthermore, transient expression of the cloned receptors in COS-1 cells, which express all the subtypes of $G_{i\alpha}$, resulted in the expression of receptors that had the same pharmacological characteristics as receptors in the CHO cells but that did not mediate SRIF inhibition of cAMP formation. These findings suggest that the cloned SRIF receptors do not couple efficiently to adenylyl cyclase.

Furthermore, the cloned SRIF receptors do not couple efficiently to phospholipase C to mediate stimulation of phosphatidylinositol turnover, as indicated from the findings of Yamada *et al.* (23) and Meyerhof *et al.* (34). SRIF receptors endogenously expressed in brain and other tissues do couple to Ca^{2+} and K^+ channels via G proteins (9, 10), suggesting that SSTR2 receptors, which are able to couple with G proteins, may associate with these cellular effector systems. However, cell lines expressing voltage-dependent and neurotransmitter-regulated Ca^{2+} and K^+ channels that could be used to test for coupling of the SSTR2 receptor to these effector systems, such as neuroblastoma (NG108 and NIE-115), pituitary (AtT-20, GH_3, and GH_4C_1), and kidney (293 cells) cell lines, all express high levels of MK 678-sensitive SRIF receptors (2, 8, 22, 25, 30).[1] This prevents their use in testing whether the cloned receptors couple to ionic conductance channels.

[1] T. Reisine, unpublished observations.

Although the SSTR2 receptor does not couple to adenylyl cyclase, it is likely to be a functionally active receptor coupled with other cellular effector systems. This is suggested by our finding that MK 678-sensitive SRIF receptors expressed in the rat striatum, which have the same pharmacological characteristics as do cloned SSTR2 receptors, do not mediate SRIF inhibition of adenylyl cyclase activity (13). However, MK 678 microinjected into rat striatum stimulates locomotor activity (16), indicating that these receptors are functionally active. Interestingly, in the striatum, SRIF inhibits adenylyl cyclase activity, suggesting that this brain region expresses a SRIF receptor subtype different from either the SSTR2 or SSTR1 receptor and indicating that there are more than two subtypes of SRIF receptors and there are likely to be more than two SRIF receptor genes.

The insensitivity of the SSTR1 receptor to pertussis toxin treatment suggests that this receptor may be different from the classical SRIF receptors reported to couple to adenylyl cyclase and Ca^{2+} and K^+ channels. Recently, a receptor has been identified that mediates the antiproliferative effects of SRIF on tumor growth in ileal mucosal and pancreatic acinar cells, via a pertussis toxin-insensitive mechanism (35). This receptor has also been shown to couple to a Na^+/H^+ ion exchanger, via a pertussis toxin-insensitive mechanism, to mediate intracellular acidification induced by SRIF (36). If this unique receptor is the SSTR1 receptor, our findings that compounds 1–3 interact selectively with the SSTR1 receptor may be of potential therapeutic importance in the treatment of cancers of the gut and pancreas.

The cloning of the SSTR1 and SSTR2 receptor subtypes provides a straightforward approach to identifying structural elements of each receptor involved in functional activity. Future studies should, therefore, be able to reveal the molecular basis for the diversity of function of the SRIF receptor subtypes.

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